OLIGONUCLEOTIDIC COMPOUNDS. XLI.*

ON THE REACTION OF RIBONUCLEOSIDE 2'(3')-PHOSPHATES WITH DIMETHYLFORMAMIDE DIMETHYLACETAL

J. SMRT

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague 6

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Adenosine 2'(3')-phosphate (*Ia*) and guanosine 2'(3')-phosphate (*Ib*) react with dimethylformamide dimethylacetal (*II*) at 20°C analogously to cytidine 2'(3')-phosphate (*Ic*), *i.e.*, under the formation of N-dimethylaminomethylene derivatives of the corresponding 2',3'-cyclic phosphates *IIIa—c*. The cyclisation does not take place with bis-tetrabutylammonium salts of nucleotides *Ia—c*.

In an earlier paper[1] of this Series, adenosine 2'(3')-phosphate (*Ia*) or guanosine 2'(3')-phosphate (*Ib*) pyridinium, ammonium, and triethylammonium salts or the free acids were claimed to react at room temperature with dimethylformamide acetals under the formation of N-dimethylaminomethylene derivatives of the starting nucleotides as the sole products. When cytidine 2'(3')-phosphate was used in the form of an ammonium salt or as a salt of a tertiary base or as a free acid, the reaction afforded N^4-dimethylaminomethylenecytidine 2',3'-cyclic phosphate. On the assumed qualitatively different behaviour of adenosine 2'(3')-phosphate and guanosine 2'(3')-phosphate a method was based[1] for the selective protection of the amino group of nucleotides *Ia,b*.

In contrast to the earlier findings[1] we have now observed in connection with other investigations that in the treatment of guanosine 2'(3')-phosphate (*Ib*) triethylammonium salt with the acetal *II*, the reaction mixture contains after one hour at room temperature 25% of N-dimethylaminomethyleneguanosine 2',3'-cyclic phosphate. After four days at room temperature, the mixture was processed with dimethoxytrityl chloride and then with aqueous ammonia to remove the dimethylaminomethylene group: 5'-dimethoxytritylguanosine 2',3'-cyclic phosphate was isolated as the single product. Additional experiments have shown that ammonium, pyridinium, and triethylammonium salts as well as the free 2'- or 3'-phosphates of cytidine, adenosine, and guanosine are cyclised on treatment with the acetal *II* with an equal rate to give after 20 hours at room temperature about 50% of the cyclic phosphate (identified

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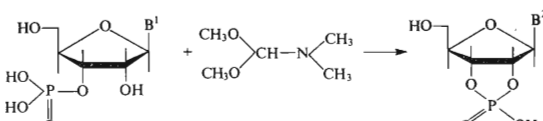
both on chromatography and electrophoresis). In all the above cases, the rate is determined by the basicity of the acetal *II*. On the other hand, the bis-tetrabutylammonium salt of cytidine 2′(3′)-phosphate does not afford even a trace of the 2′,3′-cyclic phosphate after the reaction period of three days[1]. The mono-tetrabutylammonium salt of adenosine 2′(3′)-phosphate affords after three days 5% of the 2′,3′-cyclic phosphate.



Ia, B^1 = adenine residue
Ib, B^1 = guanine residue
Ic, B^1 = cytosine residue

IIIa, B^2 = N^6-dimethylaminomethyleneadenine residue
IIIb, B^2 = N^2-dimethylaminomethyleneguanine residue
IIIc, B^2 = N^4-dimethylaminomethylenecytosine residue

As shown by the above results, the activation of the phosphoryl group and the subsequent cyclisation to give the 2′,3′-cyclic phosphate on treatment with the acetal *II* takes place with the protonated form of the phosphate. Consequently, the bis-quaternary ammonium salt does not afford any 2′,3′-cyclic phosphate and the mono-quaternary ammonium salt reacts very slowly. The nucleotides in their salts with tertiary bases are obviously protonated to a considerable extent, as indicated by the rate of cyclisation. The nature of the heterocyclic moiety does not exert any influence on the activation of the phosphoryl group at position 2′ or 3′.

EXPERIMENTAL

Descending chromatography was performed on paper Whatman No 1 in the solvent system S_1, 2-propanol–conc.NH_4OH–water (7 : 1 : 2). Paper electrophoresis was performed on the same paper dipped in tetrachloromethane; buffer solution E_1, 0.05M triethylammonium hydrogen carbonate (pH 7.5). Ultraviolet spectra were taken on a Beckman DU apparatus.

5′-O-Dimethoxytritylguanosine 2′,3′-Cyclic Phosphate

A mixture of guanosine 2′(3′)-phosphate (2 mmol), dimethylformamide (8 ml), and dimethylformamide dimethylacetal (4 ml) was shaken at room temperature. Samples were withdrawn, diluted with two volumes of concentrated aqueous ammonia, kept at room temperature for 5 h, and chromatographed in S_1. After 4 days, the reaction mixture was evaporated at 20°C/1 Torr, the residue dissolved in a mixture of pyridine (0·5 ml) and dimethylformamide (10 ml), the solution treated with dimethoxytrityl chloride (0·7 g), and the whole kept at room temperature overnight. Concentrated aqueous ammonia (5 ml) was then added, the mixture kept for 20 h at room temperature, and evaporated under an occasional addition of pyridine. The residue was coevaporated twice with pyridine, the final residue dissolved in pyridine, and the solution added dropwise under stirring into ether (100 ml). The precipitate was collected with suction, washed with ether, and dried over phosphorus pentoxide under diminished pressure to yield 1·4 g of the

pyridinium salt of 5′-O-dimethoxytritylguanosine 2′,3′-cyclic phosphate. This product was characterised by the quantitative conversion to guanosine 2′,3′-cyclic phosphate by the action of 80% aqueous acetic acid at 0°C for 3 h.

Reaction of Dimethylformamide Dimethylacetal (*II*) with Ammonium, Pyridinium, and Triethylammonium Salts of 2′(3′)-Phosphates *Ia—c*

The corresponding salt (0·1 mmol) was shaken with dimethylformamide dimethylacetal (0·1 ml) in dimethylformamide (0·2 ml). The samples were withdrawn, kept in two volumes of concentrated aqueous ammonia for 5 hours, and chromatographed in the solvent system S_1. The ratio of the phosphate and 2′,3′-cyclic phosphate was determined from the absorbance of the eluate of the corresponding spots in 0·01M-HCl at 260 nm. Content (%) of 2′,3′-cyclic phosphates: 24—26% (after 1 h), 36—38% (2 h), 40—41% (3 h), 49—51% (20 h).

Reaction of Dimethylformamide Dimethylacetal (*II*) with Adenosine 2′(3′)-Phosphate Mono-tetrabutylammonium Salt

A solution of adenosine 2′(3′)-phosphate (0·1 mmol) in 10% aqueous tetraethylammonium hydroxide (0·1 mmol) was evaporated, the residue coevaporated with dimethylformamide, and dissolved in a mixture of dimethylformamide (0·2 ml) and dimethylformamide dimethylacetal (0·1 ml). After three days, concentrated aqueous ammonia was added, the reaction mixture kept at room temperature for 5 h, and chromatographed in the solvent system S_1. The mixture contained 5% of adenosine 2′,3′-cyclic phosphate as shown by spectrophotometrical determination of the absorbance ratio of spot eluates.

REFERENCES

1. Holý A., Chládek S., Žemlička J.: This Journal *34*, 253 (1969).

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